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DISCUSSION

INVESTIGATION INTO THE ADVISABILITY OF SUBSTITUTING AGAR FOR GELATINE AS A MEDIUM FOR THE DETERMINATION OF BACTERIAL COUNTS IN WATER ANALYSIS

By Mr. W. U. C. Baton

Printed in Journal of the American Water Works Association, Vol. 1, No. 1, March, 1914, page, 11, and presented at the Philadelphia Convention of the Association, May, 1914.

Mr. Francis D. West (*by letter*): Before attempting to discuss this question the writer wishes to quote the following from the Report of Committee on Water Supplies, American Public Health Association (*American Journal of Public Health*, December, 1913.)

> There is considerable evidence, too, to indicate that this treatment (with chloride of lime) as applied in the United States does not actually destroy all of the bacteria, but simply stuns or drugs them for a time, or in other words, has a toxic effect which prevents them, for the time being, from developing on ordinary culture media.

This has been the writer's own experience with *B. coli*. He makes tests of 1 and 10 cc. and thinks that a water to be perfectly safe should be consistently free from *B. coli* in 1 cc. and practically free in 10 cc.

MEDIA

There are four commonly used, litmus lactose agar plates, lactose bile, glucose broth (Smith) and liver broth.

For unfiltered waters the writer uses litmus lactose agar plates, getting an approximate number of *B. coli* present. They are always present in unfiltered Delaware River water.

For filtered waters the litmus lactose agar plate is too inhibitory; an enrichment is needed. Lactose bile is the one most commonly used at present. It is to be preferred over glucose for waters of questionable origin, but does have an inhibitive action. American Public Health Association Report, 1912, p. 88 says:

After numerous experiments it has been found that lactose bile medium is slightly inhibitive to *B. coli* especially in attenuated form, so that any positive tests with this medium indicate fresh or recent contamination.

Glucose will give a higher per cent of positive tests with filtered and especially with disinfected water. It is claimed for liver broth that it gives a higher number of positive tests than glucose. The writer has been working for several years with bile and glucose and his experience with liver broth is limited. At present he uses glucose entirely.

The question is which should we use?

Lactose will give a series of negative results with a filtered water when glucose will be positive, especially in 10 cc. tests.

If we find that, after the water has reached the city mains and remained in them some hours, positive tests can be obtained, should not the filter operator use the most delicate test possible for *B. coli:* The filter plant superintendent or the chemist does not want to be put in a position of stating "my tests were all negative" when some bacteriologist antagonistic to the management isolates *B. coli* from the city supply some miles from the filters. It looks in that case like a question of veracity or ability on the part of the chemist. What we need is the most delicate test possible for *B. coli* and try not so much for good records as for true records and make the true records good ones. The writer would like to have the Association bring this subject up and discuss it, as he thinks it very important to both the operator and consumer. We have passed the stage when any water is a good water; what we want is the best and it is our duty to give it.

The writer was one of the chemists who in reply to Mr. Whipple's letter protested against the substitution of agar for gelatine. He, like Mr. Baton, has not changed his opinion on the subject. It is better to make the tests on both media when possible, but if only one is to be used then keep to gelatine.

The writer's experience with agar counts for filter effluents is that they are inconsistent and that spreading colonies and overgrowths are especially noticeable. For lake waters and ponds where soil washing is practically nil, agar will probably be superior; or in cases in which the sample must be stored for a time before testing, the count on gelatine is liable to develop rapidly, while the agar count will remain lower, although the agar count will also change rapidly.

The following table shows the effect of storing at room temperature (70° F.) water that had been filtered and water that had been filtered and disinfected.

TIME	FILTERED		DISINFECTED	
	Gelatine	Agar	Gelatine	Agar
Start	96	21	30	13
1 day	770	320	630	65
2 days	950	430	800	160
3 days	1100	1040	390	290
4 days	1450	1200	495	190
5 days	1430	1300	530	250
7 days	1130	660	270	120
8 days	1000	770	150	250
10 days	700	630	62	92
11 days	910	750	96	136
13 days	600	280	160	24
14 days	240	240	38	88
3 months	10	....	14	....

The above data are taken from the tests of the Delaware River water at the Torresdale Filter Plants.

Agar counts corresponding to body temperature bacteria of *B. coli* type are greatly in the minority, the types most commonly found being soil bacteria usually spore bearing. The counts on agar ordinarily increase with the temperature; in the filtered water when the temperature is above 60° F. the number on agar is greater than that on gelatine.

Agar counts in cold weather increase with the turbidity, not so much from increased pollution, as at Torresdale heavy rains usually dilute rather than pollute the water at the intake, but from soil washings. The ratio of gelatine to agar in winter runs as high as 20 to 1; in summer the agar count exceeds the gelatine count, going as high as 2 to 1. In the filtered water before disinfection the ratio in cold weather is about 10 to 1; in warm weather as in the case of the raw water the agar count is highest.

With disinfected water when the temperature is near freezing the gelatine count is considerably higher, ranging from 2 to 1 to 6 to 1. With a rise in temperature the agar count exceeds that of the gelatine, and the agar count in the treated water is very little lower than that of the untreated water, showing that the colonies developing at body temperature are spore bearing rather than pathogenic.

The table shows the absolute lack of any relationship between the count on agar and the presence of *B. coli*. For instance in February the agar count for untreated water averaged 35 and that in the treated water 25, yet the per cent of positive tests for *coli* in 1 cc. was 39 and for 10 cc. 100, while for the treated water *coli* was absent from all tests in 1 cc. The gelatine counts were 462 and 99.

For March untreated water the agar count was 27 and for the treated water 19, the gelatine being 305 and 27. *B. coli* was present, 23 per cent and 90 per cent 1 and 10 cc. for untreated, and 0 per cent and 10 for treated. For April the counts on agar were practically the same, 17 and 14, the gelatine being 89 and 11, yet in only one case was *coli* present in the treated water, that in 10 cc., and in the untreated water it was present 43 per cent of the time in 10 cc.

Comparison of gelatine and agar 1914

	RIVER		EFFLUENT	FILTERS	FILTERED	WATER BASIN	TEMP.
January......	33,900	....	....	....	227	15	36
February.....	17,250	892	462	35	99	25	36
March........	21,900	354	305	27	27	19	35
April.........	4,950	260	89	17	11	14	51

B. coli communis

Per cent positive tests

	RIVER		EFFLUENT FILTERS		FILTERED WATER BASIN	
	1 cc.	*10 cc.*	*1 cc.*	*10 cc.*	*1 cc.*	*10 cc.*
January............	100	100	....	....	6	16
February...........	82	100	39	100	0	13
March..............	7	100	23	90	0	10
April................	8	100	3	43	0	3

Mr. W. U. C. Baton: The author has read Mr. West's discussion with interest, and is pleased to find that he is in accord with the substance of the data and ideas advanced in the paper.

The writer once thought that there might be definite relation between agar and gelatine counts which would be serviceable in the interpretation of results. He also recognized the probable value of an agar count if it had a definite relation, by itself, to the sanitary quality of a water supply. The results have practically satisfied him that such a condition is not likely to be satisfactorily demonstrated soon.

Data obtained since writing the paper do not seem to add anything. The writer believes in the use of the two counts if possible for the reason that he thinks there is an advantage, in certain instances, for a better interpretation. In any case the diagnosis must be made carefully and in accord with all the known facts. The more facts known both analytical and conditional, the better the final opinion will be. The interpretation of water analyses has certainly not reached a point where it is safe to use "Rule of Thumb" methods based on mathematical calculations.

Mr. West's findings of widely varying ratios of gelatine and agar counts and also lack of relation to *B. coli* impresses this idea a little more strongly. Doubtless others have found, or will find similar results. The question of fixed standards, within narrow limits to apply to all conditions, is going to be an equally elusive problem.

Mr. West's deduction as to the presence of spore forming organisms in water which has been treated with a sterilizing agent is of considerable interest. The writer admits that he has not studied this phase as carefully as the question deserves. He is rather skeptical on the after-growth in sterilizing processes and is not so sure that it is entirely due to spores, in certain cases which have come to his attention. There is no doubt, of course, that spores might act in this way. Just what other factors enter into this condition may not be so apparent and it is for that reason that the writer wishes to make a rather exhaustive study.

It is to be hoped that all who have data on the use and interpretation of agar and gelatine counts will not hesitate to publish them so that such data will be widely available.